Spring 2019
Volume 24: Number 2

LabLink

Director, Bureau of Laboratories
Sandip Shah, PhD, HCLD(ABB)

this issue...

Bureau Vision
The Bureau of Laboratories is a stronger, more diverse team within an integrated public health system. We utilize advanced technology and innovative leadership to provide comprehensive public health services in our dynamic global community.

Bureau Mission
We are dedicated to continuing leadership in providing quality laboratory science for healthier people and communities through partnerships, communication and technical innovation.

CAP ACCREDITED

MDHHS Michigan Department of Health & Human Services

MDHHS BOL Offers Free Packing and Shipping Classes

Author: Shannon Sharp, MT(ASCP), Bioterrorism Training Coordinator

The Packaging and Shipping class provides the participant with a comprehensive overview of Federal (DOT & USPS) and International (IATA) regulations applicable to packaging and shipping of clinical laboratory specimens.

This intermediate level course offers an understanding of terminology, packaging, marking, labeling, and documentation requirements through integration of lecture, demonstrations, group exercises, and handouts. Successful completion of this course meets the requirements for employer certification.

The first two hours of class is combined training for individuals seeking their initial certification and for participants requiring recertification. Participants who have never been certified in Packaging and Shipping of Clinical Laboratory Samples, will complete an additional 2 hours of class which includes in-depth discussion, hands-on exercises, and time for questions and answers.

If you are interested in attending the sessions listed in the adjacent table, please register through the MI-TRAIN website: https://www.train.org/mi-train

The course name is ***Packaging and Shipping of Clinical Samples.***

The course identification number is ***1062236.***

Questions? Email Shannon Sharp at sharps1@michigan.gov

Facility	Address	Date	Time
Saginaw County Department of Health Room 409	1600 N. Michigan Avenue Saginaw, MI 48602	04/12/2019	10am-2pm Recertification 10am-12pm
McLaren Flint Hospital Room: Main Conference- 1 South	401 S. Ballenger Highway Flint, MI 48532	04/19/2019	9:30am-1:30pm Recertification 9:30-11:30am
Ascension St. John Hospital Lower Level Conference Room	22101 Moross Road Detroit, Mi 48236	04/24/2019	10am-2pm Recertification 10am-12pm
MDHHS Bureau of Laboratories Room 282	3350 N. Martin Luther King Junior Boulevard Lansing, MI 48909	04/30/2019	1-5pm Recertification 1-3pm
Garden City Hospital Medical Office Building Room 3-4	6245 North Inkster Road Garden City, MI 48135	05/14/2019	10am-2pm Recertification 10am-12pm
MDHHS Bureau of Laboratories Room 282	3350 N. Martin Luther King Junior Boulevard Lansing, MI 48909	05/21/2019	1-5pm Recertification 1-3pm
Munson Medical Center D tower 3rd floor-Room 2 (2 classes offered)	1105 Sixth Street Traverse City, MI 49684	06/24/2019	1st class: 10am-noon Recertification only 2nd class 12-4pm Recertification 12-2pm
McLaren Northern Michigan Hospital Room: Hospital Library	416 Connable Avenue Petoskey, MI 49770	06/25/2019	12-4pm Recertification 12-2pm
War Memorial Administration Room 2	500 Osborn Boulevard Sault Ste. Marie, MI 49783	06/26/2019	9am-1pm Recertification 9-11am

An Unusual Case of *Corynebacterium diphtheriae* biovar Belfanti conjunctivitis

Author: Glenn Fink and Joel Blostein

Diphtheria is an ancient disease with high incidence and mortality, often characterized by epidemic waves of occurrence. The disease manifests as an upper respiratory tract illness with a sore throat, impaired speech, lymphadenitis, low-grade fever, malaise, and headache. Nasopharyngeal diphtheria is characterized with adherent localized or coalescing thick gray/green pseudomembranous lesion, and it may occasionally lead to air passage obstruction. Other severe systemic illness may occur, as well as cutaneous involvement. The causative agents mainly include toxigenic strains of *Corynebacterium diphtheriae* and *Corynebacterium ulcerans.* Non-toxigenic strains may also cause disease, including endocarditis, prosthetic infections, and pharyngitis. *Corynebacterium ulcerans* has been increasingly isolated as an emerging zoonotic agent of diphtheria from companion animals such as cats and dogs.

While the disease has nearly disappeared in countries with high socioeconomic standards, it remains endemic in some subtropical and tropical countries, and among certain ethnic groups. Colonization of the causative agents is known to occur, primarily on the skin, especially among people with poor hygienic standards. With increased global travel and in population with low levels of immunity, the presence of these organisms shows that the threat of diphtheria endures.

A 64-year-old, foreign-born, Asian female with an unknown immunization history was initially seen by an ophthalmologist for a routine diabetic eye exam. Several weeks later, the patient presented with onset of a discharge from the right eye and diagnosis of conjunctivitis. The clinical laboratory isolated a coryneform bacteria from the right eye swab which identified as *Corynebacterium diphtheriae* by MALDI-TOF. There were no reported respiratory or throat symptoms, and no skin ulcers. There were no identified respiratory illnesses among the patient's household members. The local health department was notified of the culture result through the Michigan Disease Surveillance System and the isolate was submitted to the Bureau of Laboratories for further confirmation.

At the BOL reference bacteriology unit, the isolate was sub-cultured on to trypticase soy agar with 5% sheep blood (SBA) and Tinsdale agar. After 18- 24 hours of incubation, blood agar showed small, matte, off-white opaque colonies. Gram stain showed pleomorphic gram-positive bacilli. The isolate was tested catalase positive and oxidase negative. Colonies on the Tinsdale agar were black with a brown halo around the colonies, indicating tellurite reductase and cysteinase activity. Matrix assisted laser desorption ionization-time of flight (MALDI -TOF) identified the isolate as *Corynebacterium diphtheriae* (score value of 2.3).

The isolate was forwarded to the Centers for Disease Control and Prevention (CDC) for the toxin assay. *Corynebacterium diphtheriae* exotoxin is encoded by a bacteriophage carrying the *tox* gene. *Corynebacterium diphtheriae, Corynebacterium ulcerans,* and *Corynebacterium pseudotuberculosis* are the only species able to harbor the *tox* gene and potentially produce diphtheria toxin. The demonstration of the presence of the *tox* gene from a culture isolate is essential for the confirmation of diphtheria.

By Elek gel precipitation assay, *Corynebacterium diphtheriae* biovar Belfanti was confirmed and determined to be a non-toxigenic strain. This ruled out the patient as a case of diphtheria according to the current national diphtheria case definition. While this isolate was determined to be a non-toxigenic strain of *Corynebacterium diphtheriae,* it is important to note that such strains can cause serious disease, such as cases or outbreaks of skin disease, endocarditis, and occasional mortality among homeless people, alcoholics, and intravenous drug abusers. In addition, it has been shown that there are non-toxigenic isolates harboring *tox* gene*,* which could potentially represent a reservoir for toxigenic *C. diphtheriae.*

Clearly, clinical laboratories play a vital role in identifying these organisms associated with vaccine preventable bacterial diseases using both conventional microbiology methods and cross cutting technology, such as MALDI-TOF.

Histoplasmosis

Author: Tonya Heyer, Mary Grace Stobierski, Stephanie McCracken

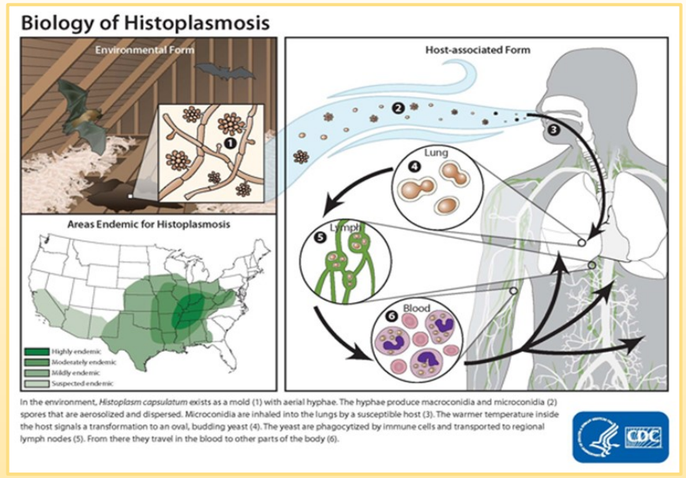
Histoplasma capsulatum is a dimorphic fungus, that is most commonly found in North and Central America but is encountered throughout the world. *Histoplasma capsulatum* is most commonly found in the central and eastern United States, in areas surrounding the Ohio and Mississippi River valleys. Soil enriched with bird or bat droppings are considered potential sources for the growth for *Histoplasma capsulatum*.

Due to the dimorphic nature, the organism exists as both a mold and yeast phase. In the environment, Histoplasma *capsulatum* exist as a mold and produces spores (arthroconidia). The initial primary infection occurs in the lung after inhalation of fungal spores, which are rapidly converted into yeast at the mammalian host body temperature. The yeast form may travel from the lung to the lymph nodes and carried in the bloodstream to other regions of the body.

Risk and Prevention:

The most common route of infection is inhalation. Humans are potentially infected due to environmental exposure with soil enriched with *Histoplasma capsulatum*. It is most commonly encountered during recreational outdoor activities. Histoplasmosis is non-contagious, but in extremely rare cases the infection has been documented in organ transplant recipients. Histoplasmosis confers partial immunity; hence the severity of illness is low on repeated exposure. Latent infections are common in an immunocompromised host, as these organisms remain sequestered for years.

Histoplasmosis has been reported in cats, however no zoonotic transmission has been documented.



In the environment, *Histoplasm capsulatum* exists as a mold (1) with aerial hyphae. The hyphae produce macroconidia and microconidia (2) spores that are aerosolized and dispersed. Microconidia are inhaled into the lungs by a susceptible host (3). The warmer temperature inside the host signals a transformation to an oval, budding yeast (4). The yeast are phagocytized by immune cells and transported to regional lymph nodes (5). From there they travel in the blood to other parts of the body (6).

Symptoms for histoplasmosis in pets include cough, lethargy, and weight loss. No symptoms have been reported in birds, even though they are considered carriers.

Symptoms:

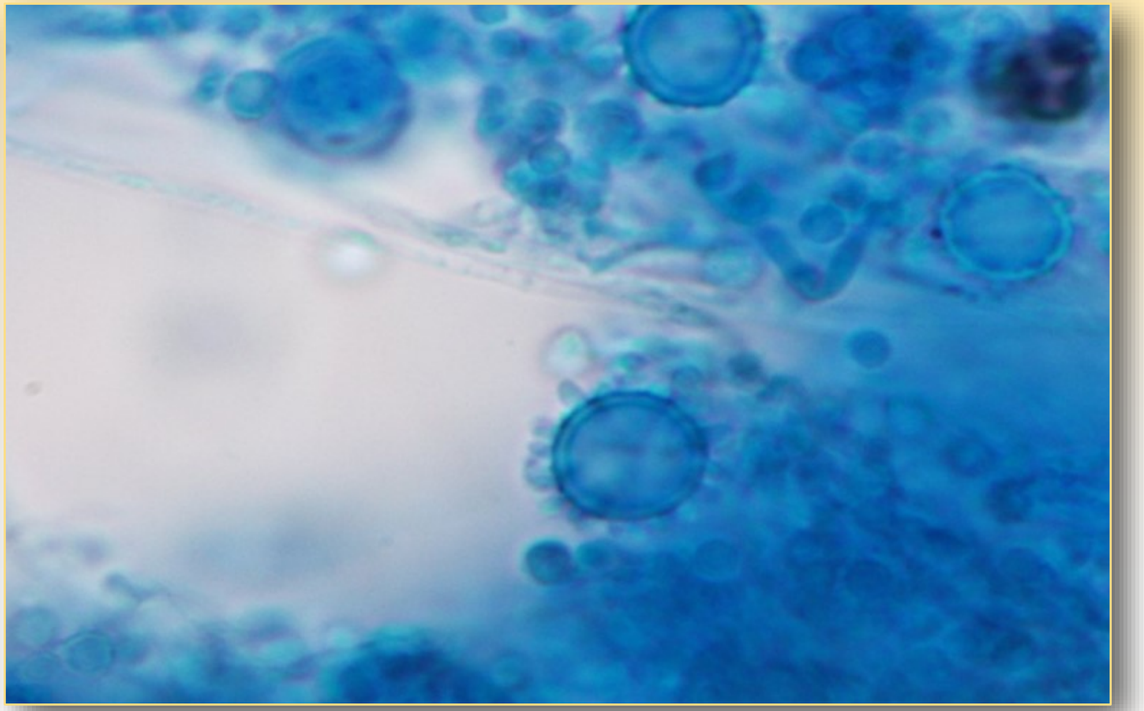
People can become infected with *Histoplasma capsulatum* after inhaling fungal spores. Infected individuals develop flu-like symptoms including fever, cough, fatigue, chills, headache, chest pain, and body aches. In most cases, the infection is self-limited. Severe histoplasmosis can occur in individuals with weakened immune systems. It is impractical to avoid exposure to fungal spores in endemic areas. People with weakened immune systems should avoid activities that involve disturbing soil enriched with bird or bat droppings, cleaning chicken coops, and exploring caves. Severe histoplasmosis can develop into a long-term lung infection or spread from the lungs to other areas of the body such as the central nervous system.

Testing:

BOL offers two methods for identifying *Histoplasma capsulatum*. Specimens are tested by the TB/Mycology unit for Fungal Identification (ID) by culture, in conjunction with the Hologic AccuProbe® DNA kit and the Viral Serology unit tests by Fungal Complement Fixation. When a specimen is suspected of having *Histoplasma capsulatum,* the culture is incubated at 30 ± 2°C. Macroscopically, the organism produces white, wooly growth (mold phase). The microscopic examination of the mold phase will show hyaline septate hyphae with unicellular, hyaline microconidia, as well as thick-walled, tuberculated macroconidia. When incubated at 36 ± 1°C, it produces white, creamy growth (yeast phase). The microscopic examination of the yeast phase shows small, oval, budding yeasts. The identification of *Histoplasma capsulatum* is confirmed in yeast phase using the AccuProbe® DNA kit. The Fungal Complement fixation assay is dependent on the ability of antibody to combine with the homologous antigen, in the presence of complement, to “fix” or utilize the complement in proportion to the strength of the antibody-antigen reaction. The strength of this reaction is measured indirectly by testing for the presence of excess complement.

continued on page 5

Histoplasmosis ...continued from page 4



Pictured: Lactophenol cotton blue staining demonstrating mold phase (septate hyphae with microconidia and tuberculated macroconidia).

Photograph by Tonya Heyer, Bureau of Laboratories

Treatment

Treatment for most people infected with histoplasmosis is not necessary. If a person is suffering from chronic histoplasmosis, severe histoplasmosis or if it has spread beyond the lungs, prescription antifungals will be needed. The most commonly prescribed antifungal medications are Itraconazole and Amphotericin B. The course of treatment can range from 3 months to 1 year depending on the patient's immune status and the severity of the infection.

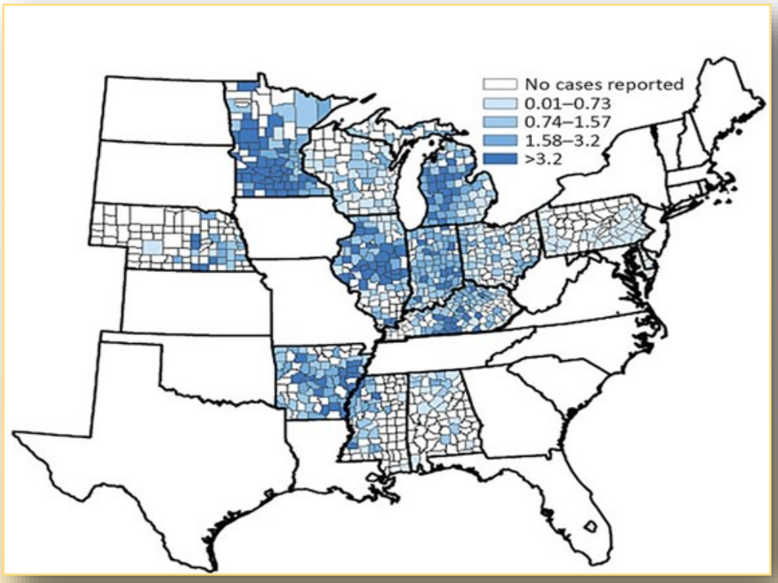
Statistics and Reporting

In the area surrounding the Ohio and Mississippi River valleys, it is estimated that 60% to 90% of people living in this area have been exposed to *Histoplasma capsulatum*. The incidence of histoplasmosis in the U.S. is calculated to be 3.4 cases per 100,000 population.

Human cases of Histoplasmosis are considered a reportable condition in the state of Michigan. Healthcare providers and laboratories are required to report diagnosed and suspected cases of histoplasmosis to their local health department and to the Michigan Department of Health and Human Services (MDHHS).

Enhanced Surveillance

The CDC is conducting an ongoing Enhanced Surveillance Project for Histoplasmosis. MDHHS is participating in the project along with 10 other public health agencies. The project includes telephone interviews with patients that have confirmed or probable histoplasmosis. The information collected during this process will include demographics, symptoms, duration, exposure, treatment, outcomes, and underlying medical conditions. Cultures from confirmed cases will be forwarded to CDC for further analysis. The information gathered in this project will be used to promote awareness about Histoplasmosis.



MALDI-TOF and Optochin Susceptibility Test to Differentiate *S. pseudopneumoniae* from *S. pneumoniae*

Author: Kaycee Hine

Streptococcus pneumoniae is one of the leading causes of community acquired pneumonia and is associated with bacteremia, meningitis, otitis media, and sinusitis. It is a member of the *Streptococcus mitis* - *Streptococcus oralis* group of viridans streptococci, which includes *S. mitis*, *S. oralis, S. cristatus, S. infantis,* and *S. peroris*. The differentiation of *S. pneumoniae* from other alpha-hemolytic streptococci include bile solubility and optochin (ethyl hydrocupreine hydrochloride) disc susceptibility test. In 2004, Arbique *et.al* identified and designated the new species "*S. pseudopneumoniae*", which closely resembles pneumococcus by its growth characteristics on sheep blood agar. They are resistant to optochin in 5% CO_2 incubation, but susceptible in ambient air. *Streptococcus pseudopneumoniae* is now considered an emerging pathogen in lower respiratory tract infections and rarely occurs in sepsis. Although *S. pneumoniae* and *S. pseudopneumoniae* share many common features, appropriate key phenotypic tests are needed to rule out *S. pseudopneumoniae* from pneumococcus and mitis group.

In February 2019, the BOL reference bacteriology unit received a blood culture isolate on a trypticase soy agar slant. Gram stain showed gram positive cocci in pairs and short chains. The isolate was sub-cultured on to trypticase soy agar with 5% sheep blood, MacConkey agar, and chocolate agar. The plates were incubated at 35ºC with 5% CO_2. After 18-24 hours of incubation, blood agar showed α hemolytic, smooth, circular colonies. On chocolate agar, the colonies were small, grey to green colonies with a zone of α hemolysis; no growth was demonstrated on MacConkey agar (Figure 1). Oxidase and catalase were negative. Matrix assisted laser desorption ionization-time of flight (MALDI-TOF) spectra was identified as *S. pseudopneumoniae* (score value 2.3) and *S. pneumoniae* (score value 2.1). The bile solubility test was negative.

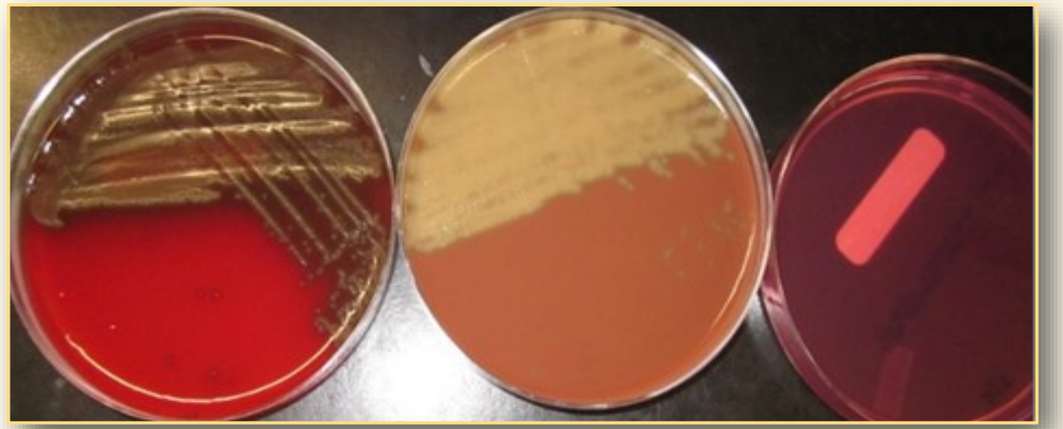


Figure 1: *α-hemolytic, smooth, circular colonies on sheep blood agar and chocolate agar. No growth on MacConkey agar.*

As described by Mohammadi et.al, the optochin test (Figure 2) was performed to differentiate *S. pseudopneumoniae* and *S. pneumoniae.*

Organism	Optochin (5µg) susceptibility	
	Ambient air	5% CO_2
S. pneumoniae	Not applicable	Susceptible
S. pseudopneumoniae	Susceptible	Resistant
Unknown clinical isolate	Susceptible	Resistant

Figure 2: *The isolate was susceptible to optochin in ambient air and resistant under 5% CO_2 incubation.*

continued on page 7

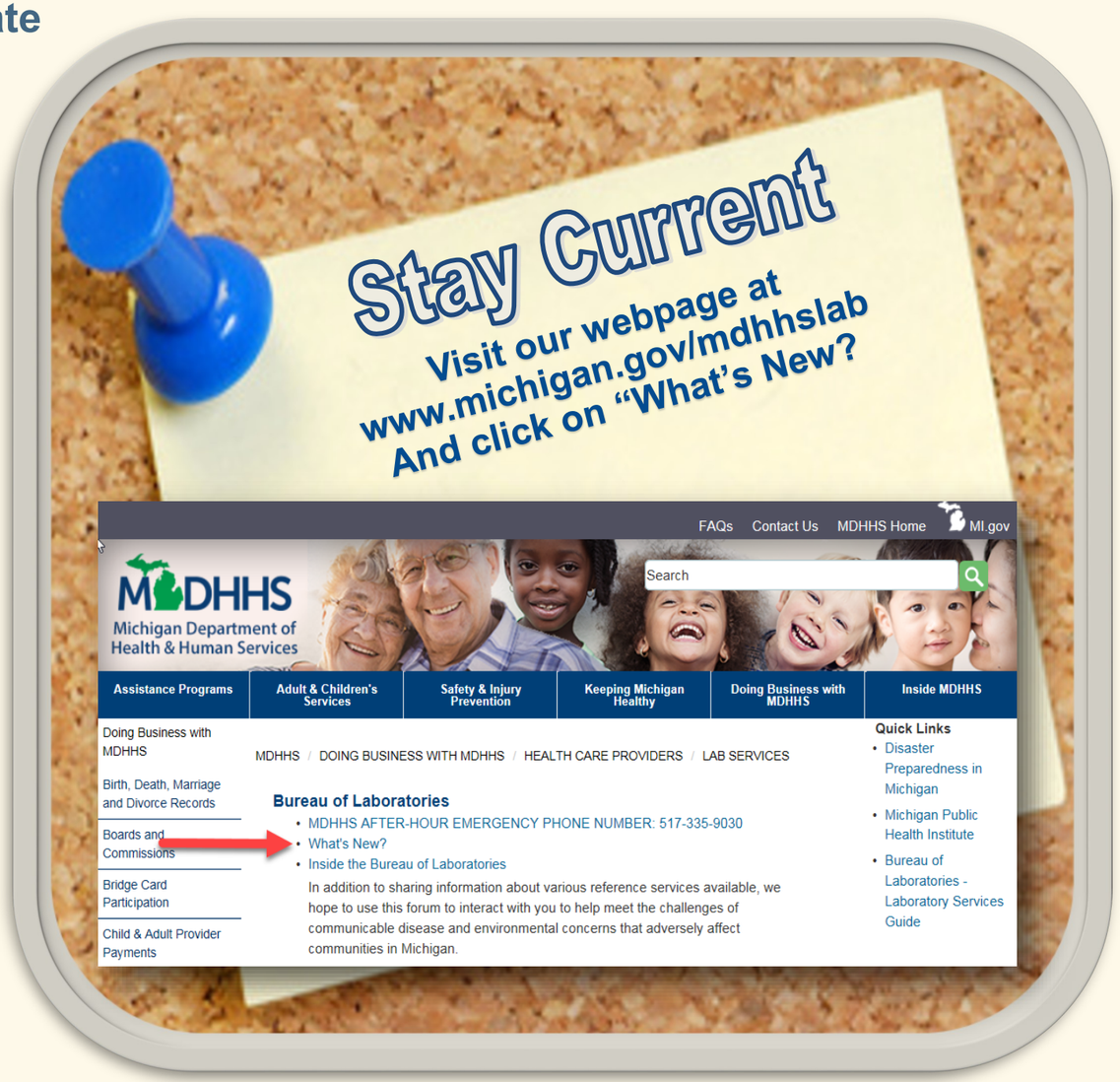
MALDI-TOF and Optochin Susceptibility Test to Differentiate *S. pseudopneumoniae* from *S. pneumoniae*

...continued from page 6

Based on colony morphology, *S. pseudopneumoniae* closely resembles pneumococcus, however, they can be differentiated by optochin susceptibility testing. *Streptococcus pseudopneumoniae* isolates are susceptible to optochin when incubated in ambient air, but resistant in 5% CO_2. To rule out pneumococcus, most hospital clinical microbiology laboratories perform optochin susceptibility testing using 5% CO_2 incubation. This creates the potential for misidentifying the optochin resistant isolates as oropharyngeal flora. *S. pseudopneumoniae* is most commonly isolated from respiratory specimens and is associated with chronic obstructive pulmonary disease (COPD), aspiration pneumonia, and recently, in septicemic illness. Due to overlapping MALDI spectra, additional phenotypic tests such as optochin susceptibility under ambient air must be performed to further rule out pneumococcus from *S. pseudopneumoniae.*

References:

- Arbique et.al., Accuracy of phenotypic and genotypic testing for identification of Streptococcus pneumoniae and Description of Streptococcus pseudopneumoniae sp. nov. J Clin Microbiol 2004; 42(10): 4686.
- Slotved HC, Facklam RR and Furrsted K. Assessment of a novel bile solubility test and MALDI–TOF for the differentiation of Streptococcus pneumoniae from other mitis group streptococci. Nature Scientific Reports 2017; 7: 7167
- Mohammadi JS and Dhanashree B. S. pseudopneumoniae: an emerging respiratory pathogen. Ind J Med Res 201; 136 (5): 877-880.



LabLink is published quarterly by the Michigan Department of Health and Human Services, Bureau of Laboratories, to provide laboratory information to Michigan health professionals and the public health community.

MDHHS is an Equal Opportunity Employer, Services, and Programs Provider.

Editor: Teresa Miller